

## Certificate of Analysis

**MAP Kinase 2/Erk2, active**  
**(Recombinant enzyme expressed in *E.Coli* cells)**  
**Item # 14-550, 14-550-K, 14-550M**  
**Parent Lot # D8MN017U**

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

**Product Description:** N-terminal GST tagged, recombinant, full-length, human MAP Kinase 2/Erk2, expressed in *E.Coli* cells. Purified using glutathione agarose. Activated with MEK1 (cat# 14-421) and repurified using Ni<sup>2+</sup>/NTA agarose. Purity 97.4% by SDS-PAGE and Coomassie blue staining. MW = 67.8kDa.

**Formulation:** 1.195mg/ml of enzyme in 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 0.03% Brij-35, 50% glycerol, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Liquid at -20°C.

**Specific Activity (Parent lot# D8MN017U):** 1410U/mg, where one unit of MAP Kinase 2/Erk2, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

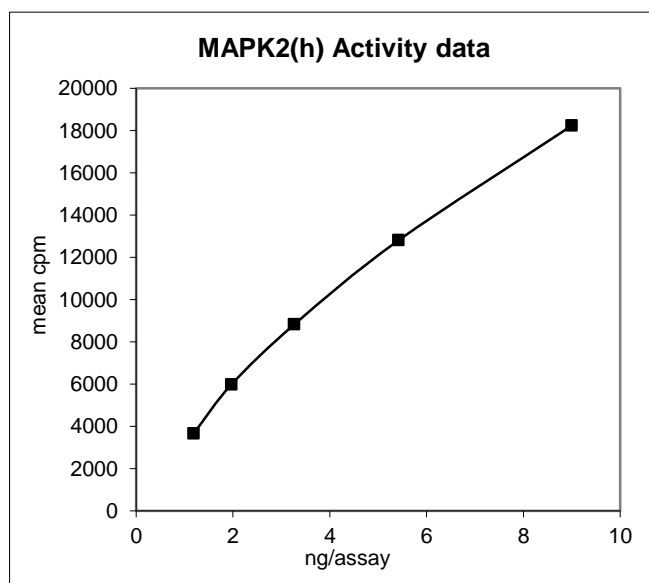
**Storage and Stability:** On receipt of material store at -20°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**FOR IN VITRO RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS OR ANIMALS**

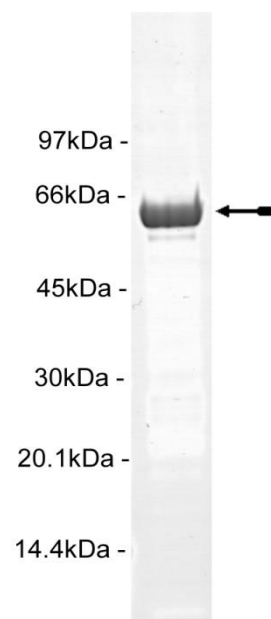
### Quality Control Testing

Kinase Assay: 1.2–9.0ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.

MS Tryptic Fingerprint: Confirmed identity as MAP Kinase 2/Erk2 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MAP Kinase 2/Erk2, active.



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### Kinase Assay Protocol

#### Stock Solutions:

- 1. 5 X Reaction Buffer:** 250mM Tris/HCl pH7.5, 0.1mM EGTA.
- 2. Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Add 2.5µl stock per assay point.
- 3. MAP Kinase 2/Erk2, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na<sub>3</sub>VO<sub>4</sub>, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.2–9.0ng per assay point.
- 4. [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells
2. Add 2.5µl of **myelin basic protein**.
3. Add **2.5µl (1.2–9.0ng) MAP Kinase 2/Erk2, active per assay point**.
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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### MAP Kinase 2/Erk2 Sequence Information

<b><u>Protein</u></b>	Human MAP Kinase 2/Erk2
<b><u>Tags</u></b>	N-terminal GST
<b><u>Native sequence</u></b>	M227 of the recombinant protein is equivalent to M1 of human MAP Kinase 2/Erk2
<b><u>Accession number</u></b>	GenBank NM_002745 (amino acid sequence only)

#### Recombinant MAP Kinase 2/Erk2 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLSSKYIA WPLQGWQATF GGGDHPKSD LVPRGSM AAAAAGAGPEMV
241 RGQVFDVGPR YTNLSYIGEG AYGMVCSAYD NVNKVRVAIK KISPFHQTY CQRTLREIKI
301 LLRFRHENII GINDIIRAPT IEQMKDVYIV QDLMETDLYK LLKTQHLSND HICYFLYQIL
361 RGLKYIHSA N VLHRDLKPSN LLLNNTCDLK ICDFGLARVA DPDHDHTGFL TEYVATRWYR
421 APEIMLSKSG YTKSIDIWSV GCILAEMLSN RPIFPGKHYL DQLNHILGIL GSPSQEDLNC
481 IINLKARNYL LSLPHKNKVP WNRLFPNADS KALDLLDKML TFNPHKRIEV EQALAHPYLE
541 QYYDPSDEPI AEAPFKFDME LDDLPEKELK ELIFEETARF QPGYRS

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#### Recombinant MAP Kinase 2/Erk2 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaacaa aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgctc aagatcgtt atgtcataaa
421 acatatttaa atggtgatca tgaacccat cctgacttca tgttgatga cgctcttgat
481 gttgttttat ataggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
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601 tggcctttgc agggctggca agccacggtt ggtggtggcg accatcctcc aaaatcggat
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1501 tggacaggtg tgttccaaa tgctgactcc aaagctctgg atttactgga taaaatgttg
1561 acatttaacc ctcaagag gattgaagtt gaacaggctc tggcccacc atacctggag
1621 cagtattatg acccaagtga tgagccatt gctgaagcgc cattcaagtt tgacatggag

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## Certificate of Analysis

1681 ttggacgact tacctaagga gaagctcaaa gaactcattt ttgaagagac tgctagattc  
1741 cagccaggat acagatctta a

Reviewed and approved by site quality representative.

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